BIOLOGICAL ASSAY AND ANTIMITOTIC ACTIVITY OF SYNTHETIC DERIVATIVES OF PODOPHYLLOTOXIN

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ABSTRACT

The antimitotic activities of several synthetic derivatives of podophyllotoxin have been determined by onion-root tip method. The probable modes of biological action of these antimitotic agents have been discussed.

INTRODUCTION

PODOPHYLLOTOXIN (1) and several of its analogues and derivatives are cytotoxic spindle poisons[1], and have received considerable attention as antitumor agents, some at clinical level[2]. Most of these compounds contain a transfused highly strained γ-lactone system[3], a feature that correlates with the smooth isomerization of (1) to its thermodynamically stable *cis* epimer, picropodophyllin[4] (2). The biological activity of (2) as well as of other *cis* analogues is very much lower than that of the *trans*-isomer[5]. β-Apopicropodophyllin (3), a dehydration product[6] of (1), contains a *cis*-fused lactone system and acts as a much stronger antimitotic agent[5]. The positive antimitotic activities of delactonized products from (1) indicate that lactone ring is not needed for antimitotic activity in this class of compounds[7].

Surveying the literature on the structure-activity relationship among the podophyllotoxin derivatives has revealed that no effort has been directed towards the study of the effect of increasing the size of the five-membered lactone ring in (1) on the antimitotic activity of (1). Also, no work on the effect of increasing the ring size of hydroaromatic ring B to the seven-membered ring has been observed. With this in view, we have synthesized[8] some derivatives of both (1) and (3) and studied their antimitotic activity by onion root tip method[9].

MATERIALS AND METHODS

Sample solution was prepared by dissolving known weight of synthetic derivative in 3 ml of absolute ethanol and diluted with distilled water to 250 ml in a standard flask.

After removal of the old roots, onion base was immersed to an extent of about 0.5 cm in a sample tube (ca 7 × 3 cm) containing the sample solution and the immersion continued for two days for germination. After two days, the germinated root tips were removed and placed in the sample tube containing the fixing solvent (ethanol-acetic acid: 3:1 v/v). After 24 hr, the fixing solvent was decanted and the root tips were washed with the preservating solvent (70% alcohol) and kept immersed in the same. An onion was also allowed to germinate in a *control* solution of absolute ethanol (3 ml) diluted with distilled water (250 ml). Root tips were placed on a clean watch glass containing staining solution (Orcein in acetic acid- 0.2 N HCl: 7:1 v/v) and heated on the flame until fumes came out. It was then cooled to room temperature. Root tips were placed on the microslide, a drop of stain solution was added and the root tips were squashed by a blade. The slide was next mounted for observation under a microscope. The total number of cells, and the number of dividing cells were counted. The per cent of the number of dividing cells compared to the control and the per cent inhibition of mitosis by the sample at a given concentration against a control were calculated. A graph of concentration versus per cent inhibition for each test compound was drawn. The concentration needed for 50% inhibition (ID_{50}) was extrapolated from the graph according to the method of Hakala *et al*[10]. ID_{50} values for the synthetic derivatives for antimitotic activity are tabulated in table 1.

MODES OF BIOLOGICAL ACTION

One of the possible modes of action could be that the strained lactone system of podophyllotoxin (1) or β-apopicropodophyllin (3) act to acylate a critical cell constituent on an N–H, SH or OH function thereby blocking the function of such a cell constituent. Chemically, the acylation process would be favoured by the removal of the lactone strain. Biochemically the acylation could destroy the activity of an essential cell constituent[11,12]. Another possibility is that the synthetic product (1) or (3) may act on a cell constituent

Table 1

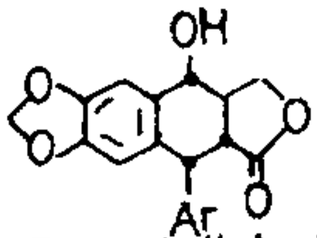
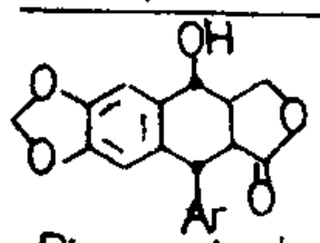
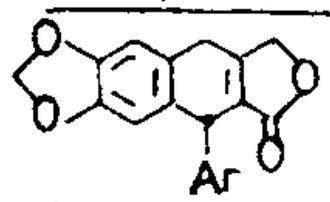
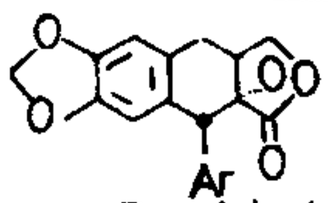
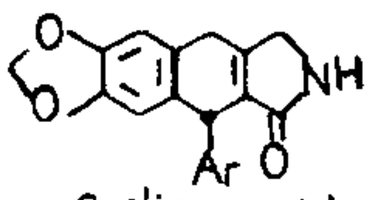
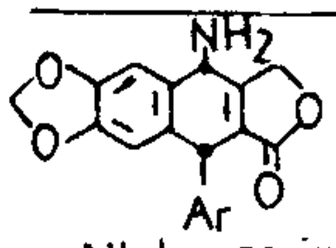
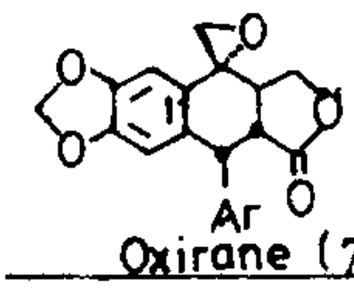
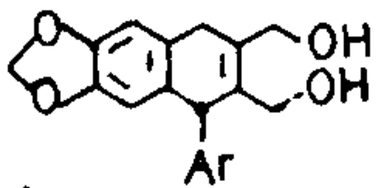
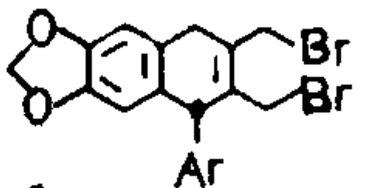
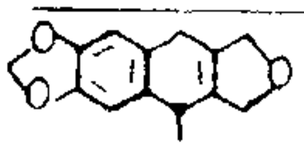
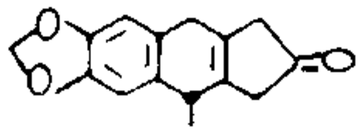
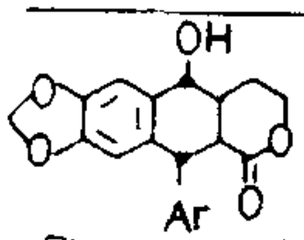
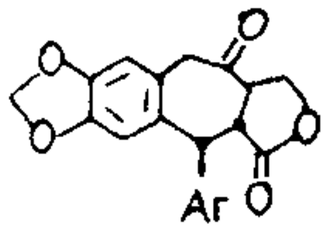
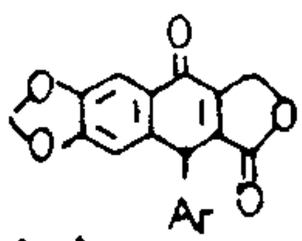
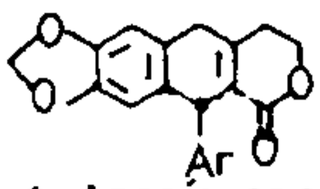
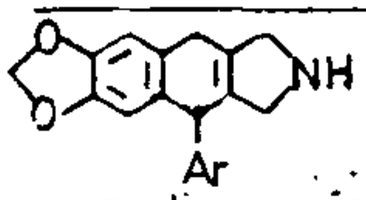
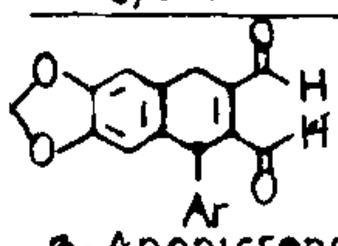
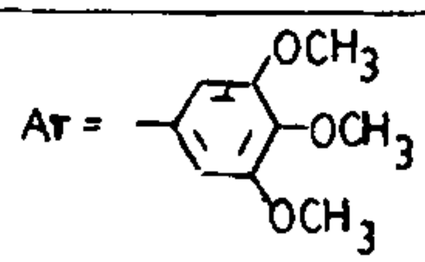
Compound	Conc (X 10^{-6} moles)	% Dividing Cells	% Dividing Cell compared to Control	% Inhibition compared to Control	ID_{50} (moles/L)
Control	—	7·78	100	0	—
 Podophyllotoxin (1)	2·90	4·33	55·7	44·3	$3·7X10^{-6}$ M
	1·45	5·30	68·2	31·8	
	4·35	3·50	45·0	55·0	
 Picropodophyllin (2)	3·86	5·92	76·2	23·8	$16·0X10^{-6}$ M
	4·35	5·90	76·0	24·0	
	6·76	5·56	71·5	28·5	
 (3) β-Apopicropodophyllin	2·53	3·91	50·3	49·7	$2·6X10^{-6}$ M
	3·54	3·27	42·0	58·0	
 Epoxide (4)	4·85	1·07	13·8	86·2	$1·0X10^{-6}$ M
	8·74	0·58	7·4	92·6	
 Cyclic amide (5)	4·06	2·73	35·0	65·0	$1·4X10^{-6}$ M
	2·03	3·10	40·0	60·0	
 Allylic amine (6)	3·41	2·78	35·7	64·3	$1·7X10^{-6}$ M
	6·81	0·70	9·0	91·0	
 Oxirane (7)	2·35	4·43	56·9	43·1	$3·9X10^{-6}$ M
	4·70	3·61	46·4	53·6	
 (8) β-Apopicropodophyllol	6·0	4·48	57·6	42·4	$8·2X10^{-6}$ M
	9·0	3·67	47·3	52·7	
 (9) β-Apopicropodophyl dibromide	3·42	2·91	37·4	62·6	$2·6X10^{-6}$ M
	4·18	1·94	24·8	75·2	

Table 1 (*Contd.*)

 Cyclic ether(10)	1·57	4·91	63·2	36·8	$2·9X10^{-6}$ M
	2·09	4·60	59·2	40·8	
 Cyclic ketone(11)	3·83	3·94	50·7	49·3	$4·0X10^{-6}$ M
	5·63	3·17	40·7	59·3	
 Picropodophyllin homolactone (12)	4·21	4·13	53·5	46·5	$4·6X10^{-6}$ M
	6·07	3·12	40·2	59·8	
 (13) Podophyllohomotoxone	5·22	4·19	53·9	46·1	$9·4X10^{-6}$ M
	4·23	4·23	54·4	45·6	
 β-Apopicropodophyllone (14)	3·90	5·21	67·0	33·0	$10X10^{-6}$ M
	6·83	4·52	58·1	41·9	
 β-Apopicropodophyllin homolactone (15)	9·76	2·62	33·7	66·3	$6·5X10^{-6}$ M
	6·83	3·82	49·0	51·0	
 Cyclic amine	16·28	1·69	21·7	78·3	$4·75X10^{-6}$ M
	8·40	2·61	33·6	66·4	
 β-Apopicropodophyllyl dialdehyde (16)	2·53	5·57	71·6	28·4	$41·0X10^{-6}$ M
	8·08	5·42	69·7	30·3	

Ar = 

not by covalent bond formation but by a non-covalent combination. Many examples of neutral compounds such as steroids binding to proteins by hydrophobic forces are known[13].

There has been growing evidence for the speculation that spindle poisons such as colchicine, vinblastine and podophyllotoxin which are potent antimitotic agents act by destroying the function of microtubules which

constitute the spindle in the cell[1]. It has been demonstrated that these spindle poisons bind non-covalently to the tubulin, the protein building block of microtubules, and that this binding inhibits mitosis in the cell. Whatever the mechanism, if non-covalent binding process occurs, a working hypothesis may be taken to be that the general overall shape of (1) derivatives is important. Should the non-lactone derivatives synthesized in the present work be anti-mitotic, the acylation mechanism would no longer be tenable. Another possibility that cannot be ruled out is that covalent bonding does occur, but it does so through a mechanism that is not apparent, particularly in non-lactonic derivatives derived from compound (3), whereas those derivatives with substituents such as nucleophilic amino group or an electrophilic epoxide ring might act through covalent bonding with some critical cell constituent.

RESULTS AND DISCUSSION

The antimitotic activity data now determined by using the onion-root tip method puts compounds (3), (1) and (2) in the same relative order of activities and the fact that (3) being the most active and (2) being the least active is consistent with the previously found relative order of activities for these three compounds by using P-815 mastocytoma cell cultured test[5]. Hence the onion-root tip method of finding the antimitotic activity should be quite valid and reliable for the determination of the antimitotic activities of the synthesized derivatives.

It is interesting and significant to note from the data in table 1 that some of the synthetic products have increased antimitotic activity, notably the epoxide (4), cyclic amide (5), allylic amine (6) with ID_{50} of 1.0×10^{-6} M, 1.4×10^{-6} M and 1.7×10^{-6} M, respectively, compared to the parent compound (3) with a ID_{50} of 2.5×10^{-6} M. The present observation that (4) is about two and a half times more active than the parent (3) is well supported by the earlier observation[14] that certain naturally occurring epoxides are strongly antitumor. It is believed that these epoxide centres are highly susceptible centres attacked by nucleophilic thiol groups in cell constituents[14]. Further, the oxirane (7) [$ID_{50} = 3.9 \times 10^{-6}$ M] is as strongly active as (1) [$ID_{50} = 3.7 \times 10^{-6}$ M], while the parent compound podophyllotoxone the precursor for the (7) is an inactive compound. Incorporation of aminogroup in the derivative such as (5) and (6) has increased the biological activity of the parent compound (3).

The β-apopicropodophyllol (8) though has very much decreased activity ($ID_{50} = 8 \times 10^{-6}$ M), the dibromide (9) is almost as active as the parent lactone (3), though the dibromide is devoid of any lactone function in it. This corroborates well with the earlier observation[7] that lactone ring system in (1) is not needed for biological activity. This is further evidenced from the observation that cyclic ether (10) [$ID_{50} = 2.9 \times 10^{-6}$ M] is as active as the lactone (3) and the cyclic ketone (11) is also quite active, though with reduced activity ($ID_{50} = 4.0 \times 10^{-6}$ M).

It is very interesting to note that the picropodophyllin homolactone (12) is a much stronger antimitotic ($ID_{50} = 4.6 \times 10^{-6}$ M) than (2) ($ID_{50} = 16 \times 10^{-6}$ M). Possibly the increase of D ring size of (2) forces the pendent ring C to assume a more axial conformation that is needed for the antimitotic activity of the derivative. This is supported by the earlier observation that (2) exists in two conformations, one resembling that of (1) which is believed to be biologically active and another conformation different from that of (1) which is believed to be inactive. On the other hand, increase of the hydroaromatic ring B size as in the podophyllohomotoxone (13) causes decrease in the antimitotic activity by nearly 3 folds, possibly the higher flexibility of the seven-membered ring B causes the change in conformation of the pendent ring C from axial to quasiaxial thereby decreasing the activity of the seven membered ketone (13). Oxygenation of the ring B as in β-apopicropodophyllone (14) decreases the activity by nearly 3 folds. This is consistent with the earlier observation that podophyllotoxone (relative activity 0.2) has drastically reduced activity compared to the deoxypodophyllotoxin (relative activity 100).

CONCLUSIONS

Our work on synthetic derivatives has shown that some have greater activity compared to the parent compounds, and some have lesser activity. The fact that many have retained their activity despite the absence of lactone ring is not compatible with the hypothesis that biological activity involves acylation. Now it is again established that the lactone ring is not needed for the antimitotic activity in (3). Therefore this observation supports the earlier view that lactone ring in (1) is not essential for biological activity[7]. It is further shown that functionalization of the hydroaromatic ring B of (3) with groups such as primary amino group or an epoxide ring (flanking the ring B and the lactone ring), enhances the antimitotic activity of the parent compound (3).

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ANNOUNCEMENT

NATIONAL SYMPOSIUM ON CONSERVATION AND USE OF PRIMATES IN MEDICAL RESEARCH

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